CLAIMS

1. A method for analyzing the organismic complexity of a sample, comprising:

a) providing a sample containing one or more organisms;

b) isolating the DNA from the organisms in the sample;

c) contacting the DNA with a fragmenting enzyme, said fragmenting enzyme being a type II restriction endonuclease, under conditions appropriate for substantially complete digestion of the DNA thereby generating a plurality of DNA fragment species, each having complementary cohesive termini;

d) incubating the DNA fragment species of step c) with a molar excess of a capture adapter, the capture adapter being a substantially duplex DNA having a portion which is covalently modified with a first member of a specific binding pair and also having one cohesive end compatible with the cohesive termini generated by the fragmenting enzyme of step c), under conditions appropriate for ligating the capture adapter to each of the complementary cohesive termini of the DNA fragment species, thereby generating a plurality of

ligation products;

e) contacting the ligation products of step d) with an anchoring enzyme under conditions for substantially complete digestion of the ligation products, said anchoring enzyme being a restriction endonuclease having a high probability of cleaving a substantial number of DNA fragment species generated in step c) at least one time, thereby generating a plurality of digestion products which have one cohesive terminus generated by the anchoring enzyme and a portion that is covalently modified with a first member of the specific binding pair;

f) capturing the digestion products of step e) by contacting the digestion products with a solid support having an attached second member of the specific binding pair;

g) incubating the solid support and captured digestion products of step f) with a molar excess of a duplex linker having a type IIS restriction enzyme recognition sequence and one cohesive terminus compatible with termini generated by the anchoring enzyme of step e), under conditions appropriate for ligating one duplex linker to the cohesive termini of the captured digestion

products, thereby ligating a recognition sequence for a type IIS restriction enzyme to the captured digestion products;

h) incubating the ligation product of step g) with the type IIS restriction enzyme, under conditions appropriate for substantially complete digestion thereby releasing the duplex linkers, each having an appended signature tag;

i) recovering the released duplex linkers and appended signature tags;

j) incubating the recovered linkers and tags of step i) with a molar excess of an amplification adapter, the amplification adapter having one terminus compatible with the termini of the appended signature tags, the incubation being carried out under conditions appropriate for ligating one amplification adapter to each appended signature tag;

k) recovering the ligation products of step j);

l) determining the nucleotide sequence of a statistically significant number of appended signature tags to generate a listing of signature tags; and,

m) relating the listing of signature tags of step l) to DNA sequences in databases to determine the

variety and relative numbers of organisms originally present in the sample thereby analyzing the organismic complexity of the sample.

2. The method of Claim 1 wherein the amplification adapter is characterized by having a restriction enzyme recognition site specific for the anchoring enzyme of step e) and which is located near the signature tag-compatible terminus.

3. The method of Claim 1, further comprising:

n) amplifying the ligation products of step k) with a pair of primers comprising a first primer specific for the duplex linker and a second primer specific for the amplification adapter, wherein each primer is labeled with a first member of a second specific binding pair;

o) incubating the amplification products of step n) with the anchoring enzyme under conditions appropriate for complete digestion of the amplification products thereby generating a digestion product mixture of end fragment digestion products and tag fragment products;

p) capturing the end fragment digestion products of step o) by contacting the digestion product with a solid support with a solid support having an attached second member of said second specific

binding pair thereby leaving the tag fragment products in solution;

q) isolating the tag fragment products from step p);

r) ligating the isolated tag fragments of step q) to form concatemers;

s) isolating concatemers of sufficient length;

t) cloning the concatemers of step s) in a plasmid vector to form concatemer constructs;

u) transforming host cells with the concatemer constructs of step t);

v) separately culturing individual transformed host cells of step u);

w) separately isolating the concatemer constructs from the cultured cells of step v);

x) determining the sequences of a statistically significant number of concatemers of the isolated concatemer constructs of step w) to generate the listing of signature tags of step l).

4. The method of Claim 1 further comprising:

n) diluting the ligation products of step k), as needed, to generate a solution containing two or fewer individual ligation product members in a specific volume;

o) separately amplifying the individual members present in the specific volume of step n) with a pair of primers, one specific for the duplex

linker of step g) and the other specific for the amplification adapter of step j);

p) sequencing a statistically significant number of the amplified members of step o) to generate the listing of signature tags of step l).

5. The method of Claim 1 wherein the solid support and captured digestion products of step f) are incubated with anchoring enzyme under conditions to ensure substantially complete digestion prior to the ligation of step g).

6. The method of Claim 4 wherein the recovered ligation products of step k) are amplified with a pair of primers, one specific for the duplex linker of step g) and the other specific for the amplification adapter of step j), prior to the dilution of step n).

7. The method of Claim 1 wherein the anchoring enzyme is a four-base cutter.

8. The method of Claim 7 wherein the four-base cutter is selected from the group consisting of *Nla*III, *Dpn*II, *Mbo*I, *Tsp*509I, *Mse*I and *Sau*3AI.

9. The method of Claim 1 wherein the type IIS restriction enzyme is *Mme*I.

10. The method of Claim 1 wherein the duplex linker is modified with a first member of a second specific binding pair and wherein the released duplex linkers and appended signature tags are recovered in step i) by

contacting the released duplex linkers and appended signature tags with a second solid support having covalently attached a second member of the second specific binding pair.

11. The method of Claim 1 wherein the specific binding pair is selected from the group consisting of biotin/streptavidin, antigen/antibody, sugar/lectin, apoenzyme/cofactor, hormone/receptor, enzyme/inhibitor, and complementary homopolymeric oligonucleotides.

12. The method of Claim 1 wherein the solid support is selected from the group consisting of magnetic beads, glass beads, filter membranes, filter papers and polymeric beads.

13. The method of Claim 3 wherein the second specific binding pair is identical to the specific binding pair of Claim 1.

14. The method of Claim 3 wherein the second specific binding pair is different from the specific binding pair of Claim 1.

15. The method of Claim 4 wherein the sequencing in step p) is performed using capillary gel electrophoresis.

16. The method of Claim 4 wherein the dilution of step n) generates a solution having one or fewer individual product members in the specific volume.

17. The method of Claim 16 wherein the sequencing is

performed by a method selected from the group consisting of pyrosequencing and capillary gel electrophoresis.

18. The method of Claim 1 wherein the sample is an environmental sample.

19. The method of Claim 18 wherein the environmental specimen is a forensic specimen.

20. The method of Claim 1 wherein the sample is a biological specimen.

21. The method of Claim 20 wherein the biological specimen is selected from the group consisting of blood, urine, oral mucous, cervical scraping, tissue biopsy and skin.

22. The method of Claim 20 wherein the biological specimen is a forensic specimen.

23. A method for analyzing the complexity of single-stranded nucleic acid in a sample, comprising:

a) providing a single-stranded nucleic acid-containing sample;

b) isolating the single-stranded nucleic acid from the sample;

c) generating a plurality of double-stranded DNA species, by the method comprising:

i) incubating the single-stranded nucleic acid of step b) with a molar excess of oligonucleotide primer, the oligonucleotide

primer having a sequence complementary to a sequence of the single-stranded nucleic acid, the oligonucleotide primer being covalently modified with a first member of a specific binding pair, the incubation being carried out under conditions appropriate for hybridization of the oligonucleotide primer to the single-stranded nucleic acid; and

ii) contacting the hybridized product of step *i*) with a polymerase selected from the group consisting of reverse transcriptase and DNA polymerase under conditions for generating a duplex DNA copy of each hybridized single-stranded nucleic acid which duplex DNA copy is modified with a first member of a specific binding pair;

d) contacting the DNA copies of step c) with an anchoring enzyme under conditions for substantially complete digestion of the DNA copies, said anchoring enzyme being a restriction endonuclease having a high probability of cleaving a substantial number of DNA copies generated in step c) at least one time, thereby generating a plurality of digestion products which have one cohesive terminus generated by the anchoring

enzyme and a portion that is covalently modified with a first member of the specific binding pair;

e) capturing the digestion products of step d) by contacting the digestion products with a solid support having covalently attached a second member of the specific binding pair;

f) incubating the solid support and captured digestion products of step e) with a molar excess of a duplex linker having a type IIS restriction enzyme recognition sequence and one cohesive terminus compatible with termini generated by the anchoring enzyme of step d), under conditions appropriate for ligating one duplex linker to the cohesive termini of the captured digestion products, thereby ligating a recognition sequence for a type IIS restriction enzyme to the captured digestion products;

g) incubating the ligation product of step f) with the type IIS restriction enzyme, under conditions appropriate for substantially complete digestion thereby releasing the duplex linkers, each having an appended signature tag;

h) recovering the released duplex linkers and appended signature tags;

i) incubating the recovered linkers and tags of step

h) with a molar excess of an amplification adapter, the amplification adapter having one terminus compatible with the termini of the appended signature tags, the incubation being carried out under conditions appropriate for ligating one amplification adapter to each appended signature tag;

j) recovering the ligation products of step i);

k) diluting the products of step j), as needed, to generate a solution containing two or fewer individual product members in a specific volume;

l) separately amplifying the individual members present in the specific volume of step k) with a pair of primers one specific for the duplex linker of step f) and the other specific for the amplification adapter of step i);

m) sequencing a statistically significant number of the products of step l) to generate a listing of signature tags;

n) relating the listing of signature tags of step m) to sequences in databases to determine the variety and relative numbers of single-stranded nucleic acids originally present in the sample thereby analyzing the complexity of the single-stranded nucleic acid of the sample.

24. The method of Claim 23 wherein the solid support and captured digestion products of step e) are incubated with anchoring enzyme under conditions to ensure substantially complete digestion prior to the ligation of step f).

25. The method of Claim 23 wherein the recovered ligation products of step j) are amplified with a pair of primers one specific for the duplex linker of step f) and the other specific for the amplification adapter of step i) prior to the dilution of step k).

26. The method of Claim 23 wherein the single-stranded nucleic acid is RNA.

27. The method of Claim 26 wherein the RNA is of prokaryotic or viral origin.

28. The method of Claim 26 wherein the RNA is of eukaryotic origin.

29. The method of Claim 28 wherein the RNA is poly (A)+ RNA.

30. The method of Claim 29 wherein the oligonucleotide primer is oligo d(T).

31. The method of Claim 23 wherein the single-stranded nucleic acid is DNA.

32. The method of Claim 31 wherein the DNA is of viral or bacteriophage origin.

33. The method of Claim 23 wherein the oligonucleotide primer is a random oligonucleotide primer.

34. The method of Claim 33 wherein the random

oligonucleotide primers are hexamers.

35. The method of Claim 23 wherein the anchoring enzyme is a four-base cutter.

36. The method of Claim 35 wherein the four-base cutter is selected from the group consisting of *Nla*III, *Dpn*II, *Mbo*I, *Tsp*509I, *Mse*I and *Sau*3AI.

37. The method of Claim 23 wherein the type IIS restriction enzyme is *Mme*I.

38. The method of Claim 23 wherein the binding pair is selected from the group consisting of biotin/streptavidin, antigen/antibody, sugar/lectin, apoenzyme/cofactor, hormone/receptor, enzyme/inhibitor, and complementary homopolymeric oligonucleotides.

39. The method of Claim 23 wherein the solid support is selected from the group consisting of magnetic beads, glass beads, filter membranes, filter papers and polymeric beads.

40. The method of Claim 23 wherein the sequencing in step m) is performed using capillary gel electrophoresis.

41. The method of Claim 23 wherein the dilution of step k) generates a solution having one or fewer individual product members in the specific volume.

42. The method of Claim 41 wherein the sequencing is performed by a method selected from the group consisting of pyrosequencing and capillary gel electrophoresis.

43. The method of Claim 23 wherein the sample is an environmental sample.

44. The method of Claim 43 wherein the environmental specimen is a forensic specimen.

45. The method of Claim 23 wherein the sample is a biological specimen.

46. The method of Claim 45 wherein the biological specimen is selected from the group consisting of blood, urine, oral mucous, cervical scraping, tissue biopsy and skin.

47. The method of Claim 45 wherein the biological specimen is a forensic specimen.

48. A composition of matter comprising a collection of substantially duplex DNA amplification adapters of the present invention characterized by having a degenerate, ligatable overhang that is capable of hybridizing in ligatable form to ends generated upon digestion of the ligation products of step g) of Claim 1 or step f) of Claim 23 with the type IIS restriction enzyme compatible with the duplex linker of step g) of Claim 1 or step f) of Claim 23, and further characterized by having a restriction enzyme recognition site located near the overhang that is specific for the anchoring enzyme of step e) of Claim 1 or step d) of Claim 23.

49. The composition of Claim 48 wherein the amplification adapters are additionally characterized by having one or more additional restriction endonuclease recognition

sequences.

50. A method for analyzing the variety of members of specific phyla or families of organisms contained in a sample using single point genome signature tags comprising the steps of:

a) providing a sample containing one or more organisms;

b) isolating the DNA from the organisms in the sample;

c) contacting the DNA with a fragmenting enzyme under conditions appropriate for substantially complete digestion of the DNA thereby generating a plurality of DNA fragments, each having complementary cohesive termini, said fragmenting enzyme being a type II restriction endonuclease which does not cleave within conserved segments of a gene of focus, said gene of focus being a gene containing segments that are highly conserved across a phylum or a family of organisms and segments that are species-specific across the phylum or family of organisms;

d) incubating the DNA fragments of step c) with a molar excess of a duplex linker having a type IIS restriction enzyme recognition sequences and one cohesive terminus compatible with termini generated by the fragmenting enzyme of step c), under conditions appropriate for ligating one

duplex linker to each cohesive terminus of the DNA fragments thereby generating a plurality of DNA fragment-duplex linker species;

e) amplifying a portion of a specific subset of DNA fragment-duplex linker species using a pair of primers comprising a first primer specific for the duplex linker and an anchoring primer, said anchoring primer being specific for a conserved segment of the gene of focus and which anchoring primer is covalently modified with a first member of a specific binding pair, thereby generating a mixture of unamplified DNA fragment-duplex linker species and amplified portions of a subset of the DNA fragment-duplex linker species, said amplified portions comprising sequences that are conserved across the phylum or family and sequences that are species-specific and which species-specific sequences contain the single point genome signature tags;

f) capturing the amplified portions of the subset by contacting the mixture of step e) with a solid support having an attached second member of the specific binding pair;

g) incubating the solid support and captured amplified portions of step f) with the type IIS restriction enzyme, under conditions appropriate for substantially complete digestion thereby

releasing the duplex linkers, each having an appended single point genome signature tag (SP-GST);

h) recovering the released duplex linkers and appended SP-GSTs;

i) incubating the recovered linkers and SP-GSTs of step h) with a molar excess of an amplification adapter, the amplification adapter having one terminus compatible with the termini of the appended SP-GSTs, the incubation being carried out under conditions appropriate for ligating one amplification adapter to each appended SP-GST;

j) recovering the ligation product of step i);

k) determining the nucleotide sequence of a statistically significant number of appended SP-GSTs to generate a listing of SP-GSTs; and,

l) relating the listing of SP-GSTs of step k) to DNA sequences in databases to determine the variety of members of specific phyla or families of organisms contained in the sample.

51. The method of Claim 50 in which the SP-GSTs are located upstream or downstream of the gene of focus.

52. The method of Claim 50 in which the SP-GSTs are located within the gene of focus.

53. The method of Claim 50 wherein the gene of focus is selected from the group consisting of rDNA genes of archaebacteria, rDNA genes of eubacteria, rDNA genes of

eukaryotes, rDNA genes of fungi or rDNA genes of organelles.

54. The method of Claim 50 wherein the gene of focus is a gene or locus that is conserved among related organisms.

55. The method of Claim 50 wherein the gene of focus is a gene or locus encoding an enzyme of a pathway that is conserved among related organisms.

56. A method for identifying methylated CpG island-associated genome signature tags comprising:

a) providing a sample containing genomic DNA;

b) isolating the genomic DNA from the sample;

c) contacting the isolated DNA from step b) with a fragmenting type II restriction enzyme, said fragmenting restriction enzyme having a low probability of cleaving G-C rich DNA sequences, under conditions appropriate for substantially complete digestion of the DNA, thereby generating a plurality of DNA fragment species, each having complementary cohesive termini;

d) separating methylated CpG island (Me-CpGI) fragments from fragments in which CpG sequences are not methylated by contacting the products of step c) with an affinity resin that specifically recognizes and binds Me-CpG sequences thereby capturing the fragments having Me-CpGIs on the

resin;

e) incubating the captured Me-CpGI fragments with a molar excess of duplex linker having a type IIS restriction enzyme recognition sequence and one cohesive terminus compatible with termini generated by the fragmenting restriction enzyme, under conditions appropriate for ligating one duplex linker to each terminus of the captured fragments of step d);

f) contacting the ligation products of step e) with the type IIS restriction enzyme, under conditions appropriate for complete digestion of the captured Me-CpGI fragments, thereby releasing the duplex linkers and appended Me-CpGI-associated signature tags;

g) recovering the released linkers and appended tags;

h) incubating the recovered linkers and tags of step g) with a molar excess of an amplification adapter having one terminus compatible with the termini of the appended signature tags under conditions appropriate for ligation of one amplification adapter to each appended signature tag;

i) recovering the ligation products of step h);

j) determining the nucleotide sequence of a statistically significant number of appended

signature tags to generate a listing of methylated CpG Island-associated signature tags.

57. The method of Claim 56 in which the fragmenting type II restriction enzyme is selected from the group consisting of *Tsp*509I, *Mse*I, *Ase*I, *Sal*I, *Apa*LI and *Eco*RI.

58. The method of Claim 1 wherein step c) further comprises contacting the DNA with a second fragmenting enzyme, under conditions appropriate for substantially complete digestion of the DNA by the second fragmenting enzyme.

59. The method of Claim 58 in which the first fragmenting enzyme is sensitive to methylation of CpG sequences and the second fragmenting enzyme is insensitive to methylation of CpG sequences.

60. The method of Claim 59 in which the first and second fragmenting enzymes are isoschizomers.

61. The method of Claim 60 in which the isoschizomers cleave at different locations within a recognition sequence.

62. The method of Claim 61 in which the first fragmenting enzyme is *Sma*I and the second fragmenting enzyme is *Xma*I.

63. The method of Claim 58 in which the anchoring enzyme is chosen from the group consisting of *Tsp*509I, *Mse*I, *Ase*I, *Sal*I, *Apa*LI and *Eco*RI.

64. A method for determining a visualizable change in organismic complexity of a sample from one sampling

time to the next, the method comprising:

a) providing a sample containing one or more organisms;

b) isolating the DNA from the organisms in the sample;

c) contacting the DNA with a fragmenting enzyme, said fragmenting enzyme being a type II restriction endonuclease, under conditions appropriate for substantially complete digestion of the DNA thereby generating a plurality of DNA fragment species, each having complementary cohesive termini;

d) incubating the DNA fragment species of step c) with a molar excess of a capture adapter, the capture adapter being a substantially duplex DNA having a portion which is covalently modified with a first member of a specific binding pair and also having one cohesive end compatible with the cohesive termini generated by the fragmenting enzyme of step c), under conditions appropriate for ligating the capture adapter to each of the complementary cohesive termini of the DNA fragment species, thereby generating a plurality of ligation products;

e) contacting the ligation products of step d) with

an anchoring enzyme under conditions for substantially complete digestion of the ligation products, said anchoring enzyme being a restriction endonuclease having a high probability of cleaving a substantial number of DNA fragment species generated in step c) at least one time, thereby generating a plurality of digestion products which have one cohesive terminus generated by the anchoring enzyme and a portion that is covalently modified with a first member of the specific binding pair;

f) capturing the digestion products of step e) by contacting the digestion products with a solid support having an attached second member of the specific binding pair;

g) incubating the solid support and captured digestion products of step f) with a molar excess of a duplex linker having a type IIS restriction enzyme recognition sequence and one cohesive terminus compatible with termini generated by the anchoring enzyme of step e), under conditions appropriate for ligating one duplex linker to the cohesive termini of the captured digestion products, thereby ligating a recognition sequence for a type IIS restriction enzyme to the captured

digestion products;

h) contacting the ligation products of step g) with one or more restriction enzymes that are neither identical to nor isoschizomers of the fragmenting enzyme, the anchoring enzyme or the type IIS restriction enzyme, under conditions appropriate for substantially complete digestion, thereby releasing the duplex linker-terminal restriction fragments from the captured ligation products;

i) recovering the released linker-fragments of step n);

j) separating the recovered linker-fragments of step o) by gel electrophoresis;

k) recording the electrophoretic distribution of the separated linker-fragments as a Terminal Restriction Fragment Barcode (TFRB);

l) providing a second, temporally distinct sample from a location identical to the location at which the step a) sample was provided;

m) repeating steps a) through k) to generate a second TRFB; and

n) comparing the first TRFB to the second TRFB to determine if the organismic complexity changed from the first to the second sampling time.

65. The method of Claim 64 wherein the electrophoretic distribution is recorded electronically.

66. The method of Claim 65 wherein the visualizable changes

are detected by electronic comparisons of electrophoretic distributions recorded from samples taken from a single location but at different times.

67. A method for generating a listing of genome signature tags from fragmented genomic DNA comprising the steps of:

a) providing a sample containing fragmented genomic DNA;

b) isolating the fragmented DNA from the sample;

c) treating the fragmented genomic DNA under conditions appropriate for producing blunt ends on all fragments;

d) incubating the blunt ended fragments of step c) with a molar excess of a duplex linker, said linker having one duplex blunt end and a type IIS restriction enzyme recognition site located adjacent to said blunt end, under conditions appropriate for covalent ligation of the linker to the termini of the blunt ended fragments;

e) amplifying the ligation products of step d) with a primer specific for the linker, said primer being covalently modified with a first member of a first specific binding pair;

f) digesting the amplified DNA of step e) with the type IIS restriction enzyme, under conditions appropriate for substantially complete digestion

thereby generating a digestion mixture comprising duplex linkers which are modified with said first member of said first binding pair, each having an appended signature tag, and DNA fragments that are unmodified;

g) capturing the duplex linkers and appended signature tags by contacting the mixture of step f) with a solid support having an attached second member of said first specific binding pair;

h) releasing the captured linkers and appended tags from the solid support;

i) incubating the released linkers and tags with a degenerate amplification adapter, said amplification adapter having one terminus compatible with the termini of the appended signature tags and having one or more adapter-associated type II restriction enzyme recognition sequences, the incubation being carried out under conditions for ligation of one amplification adapter to each appended signature tag;

j) amplifying the ligation product of step i) with a pair of primers comprising a first primer which is specific for the duplex linker and which is modified with a first member of a second specific binding pair and a second primer which is specific

for the amplification adapter;

k) digesting the amplified product of step j) with a type II restriction enzyme specific for the adapter-associated type II restriction enzyme recognition sequences, thereby generating a digestion mixture comprising a portion of the amplification adapter and duplex linkers with appended signature tags, which linkers and tags are modified with said first member of said second binding pair and which have a cohesive terminus attached to the signature tag;

l) capturing the linkers and appended tags on a second solid support having an attached second member of said second specific binding pair; and

m) determining the nucleotide sequence of a statistically significant number of appended tags to generate a listing of signature tags from the sample containing fragmented DNA.

68. The method of Claim 67 wherein the linker additionally has one or more type II restriction enzyme recognition sites.

69. The method of Claim 67 wherein the linker is a partially duplex linker.

70. The method of Claim 69 wherein the linker is dephosphorylated at the 5' termini and wherein the

ligation products of step d) are made fully duplex prior to the amplification of step e).

71. The method of Claim 67 wherein the amplification adapter is a partially duplex Y-shaped amplification adapter.

72. The method of Claim 67 wherein the DNA of the sample is fragmented by a mechanical method.

73. The method of Claim 72 wherein the mechanical method is selected from the group consisting of nebulization, sonication and hydroshear.

74. The method of Claim 72 wherein the sample is chemically crosslinked prior to fragmentation of the DNA.

75. The method of Claim 67 wherein the first and second specific binding pairs are identical.

76. The method of Claim 67 wherein the type IIS restriction enzyme is *Mme*I.

77. The method of Claim 67 wherein one adapter-associated type II restriction enzyme recognition site is the recognition site for *Nla*III.

78. A method for generating a listing of genome signature tags from fragmented genomic DNA comprising the steps of:

a) providing a sample containing fragmented genomic DNA;

b) isolating the fragmented DNA from the sample;

c) treating the fragmented genomic DNA under

conditions appropriate for producing blunt ends on all fragments;

d) incubating the blunt ended fragments of step c) with a molar excess of a capture adapter, the capture adapter being a duplex DNA having a portion which is covalently modified with a first member of a first specific binding pair and one blunt end, under conditions appropriate for ligating the capture adapter to each of the blunt ends of the DNA fragments, thereby generating a plurality of ligation products;

e) contacting the ligation products of step d) with an anchoring enzyme under conditions for substantially complete digestion of the ligation products, said anchoring enzyme being a restriction endonuclease having a high probability of cleaving a substantial number of the DNA fragments of step c) at least one time, thereby generating a plurality of digestion products which have one cohesive terminus generated by the anchoring enzyme and a portion that is covalently modified with the first member of the first specific binding pair;

f) capturing the digestion products of step e) by contacting the digestion products with a solid support having an attached second member of the first specific binding pair;

g) incubating the solid support and captured digestion products of step f) with a molar excess of a duplex linker having a type IIS restriction enzyme recognition sequence and one cohesive terminus compatible with termini generated by the anchoring enzyme of step e), under conditions appropriate for ligating one duplex linker to the cohesive termini of the captured digestion products, thereby ligating a recognition sequence for a type IIS restriction enzyme to the captured digestion products;

h) incubating the ligation product of step g) with the type IIS restriction enzyme, under conditions appropriate for substantially complete digeston thereby releasing the duplex linkers, each having an appended signature tag;

i) recovering the released duplex linkers and appended signature tags;

j) incubating the recovered linkers and tags of step i) with a molar excess of an amplification adapter, the amplification adapter having one terminus compatible with the termini of the appended signature tags, the incubation being carried out under conditions appropriate for ligating one amplification adapter to each

60 appended signature tag;

k) recovering the ligation products of step j);

l) determining the nucleotide sequence of a statistically significant number of appended signature tags to generate a listing of signature tags.

79. The method of Claim 78 wherein the ligation products of step d) are amplified using a primer specific for the duplex linker prior to the digestion of step e).

80. The method of Claim 78 wherein the linker is a partially duplex linker.

81. The method of Claim 80 wherein the linker is dephosphorylated at the 5' termini and wherein the ligation products of step d) are made fully duplex prior to the digestion of step e).

82. The method of Claim 78 wherein the amplification adapter is a partially duplex Y-shaped amplification adapter.

83. The method of Claim 78 wherein the DNA of the sample is fragmented by a mechanical method.

84. The method of Claim 83 wherein the mechanical method is selected from the group consisting of nebulization, sonication and hydroshear.

85. The method of Claim 83 wherein the sample is chemically crosslinked prior to fragmentation of the DNA.

86. The method of Claim 78 wherein the type IIS restriction enzyme is *Mme*I.

87. A primer pair useful for generating unique genome sequence data from an organism-specific region of interest, the organism-specific region of interest being adjacent to a region which is conserved across species boundaries, the primer pair comprising:

a) a first primer of sufficient length to hybridize specifically, under conditions appropriate for polymerase chain reaction (PCR) amplification, with the region which is conserved across species boundaries; and

b) a second primer which contains a sequence of nucleotides, which is specific for the duplex linker of the present invention,

the use of the first and second primers in a PCR amplification protocol generating an amplified fragment which, when contacted with a type IIS restriction enzyme specific for the duplex linker, under conditions appropriate for digestion by the enzyme, releases a genome signature tag which can be used to identify specific organisms or can be used to prime DNA synthesis to generate unique sequence data from the organism-specific region of interest adjacent to the region that is conserved across species boundaries.

88. The primer pair of Claim 87 wherein the first primer is labeled at the 5′ terminus with a first member of a specific binding pair.

89. The primer pair of Claim 87 wherein the type IIS restriction enzyme is selected from the group consisting of *Eco*57MI and *Mme*I.

90. The primer pair of Claim 89 wherein the type IIS restriction enzyme is *Mme*I.

91. The method of any one of Claims 1, 23, 50, 56, 67, or 78 wherein the amplification adapter is part of a collection comprising amplification adapters with degenerate overhang sequences.

92. The method of any one of Claims 1, 23, 50, 56, 67, or 78 wherein the amplification adapter is part of a collection comprising amplification adapters with a subset of degenerate overhang sequences.

93. The method of any one of Claims 9, 37, 76, or 86 wherein the amplification adapter is part of a collection comprising amplification adapters with ligatable 3′ overhangs which encode any one of 4-fold, 8-fold, or 16-fold degenerate sequences.

94. The composition of Claim 48 wherein the collection comprises amplification adapters with ligatable overhangs comprising a subset of degenerate sequences.